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CHAPTER 3

Partial DAZ deletions in a family
with five infertile brothers


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Chapter 3

Abstract

Objective
To study the genetic cause of infertility in a family with five infertile brothers.

Design
Case report.

Setting
Center for reproductive medicine at a university medical center.

Patient(s)
Five brothers presenting with primary infertility due to severely impaired spermatogenesis, also their parents and two other paternally related family members.

Intervention(s)
Fluorescence In Situ Hybridisation and Sequence Family Variant analysis was performed in leukocyte DNA to determine the number of DAZ genes. Linkage analysis was performed for X chromosome inheritance, and mitochondrial DNA (mtDNA) was screened for mutations.

Main outcome measure(s)
DAZ gene copy number, X chromosome linkage, mtDNA sequence.

Result(s)
With conventional PCR analysis, no deletions of the AZFc region were found, but with FISH and SFV analysis, only two DAZ genes instead of four were detected in all individuals tested. The five brothers did not share an identical X chromosomal locus, and no mutations were found in the mtDNA of the index patient.

Conclusion(s)
A reduced copy number of the DAZ genes is found in five infertile brothers with severely impaired spermatogenesis, as well as in their normospermic father and in two other fertile paternally related family members. This illustrates that the phenotype associated with a reduced copy number of the DAZ genes can be extremely variable.
Partial DAZ deletions in a family with five infertile brothers

Introduction

The molecular aetiology of male factor subfertility due to impaired spermatogenesis is still unknown in the majority of cases. Structural and numerical chromosomal abnormalities are found in approximately 4% of patients with azoo- or oligozoospermia (Tuerlings et al., 1998). Deletions of any of the Y chromosomal regions Azoospermia Factor a, b or c (respectively, AZFa, AZFb, and AZFc) are also a frequent cause of spermatogenic defects. Deletions of the AZFc region are most frequently found, i.e. in 2-10% of azoospermic or severely oligozoospermic men (Reijo et al., 1995, 1996; Kremer et al., 1997; van der Ven et al., 1997; Simoni et al., 1997; Kuroda-Kawaguchi et al., 2001). The AZFc region spans 3.5 Mb and contains seven gene families that are all thought to be involved in spermatogenesis (Kuroda-Kawaguchi et al., 2001). One of these gene families is the DAZ (Deleted in Azoospermia) gene family, which consists of four nearly identical copies divided into two clusters with two genes each (Kuroda-Kawaguchi et al., 2001; Saxena et al., 2000). Although most deletions involve a deletion of all four DAZ genes, absence of only two of the DAZ genes also is associated with impaired spermatogenesis (de Vries et al., 2002; Fernandes et al., 2002).

Most cases of male factor subfertility are individual cases, and the genetic aberrations found are usually de novo events. However, clustering of male subfertility in families has been described (Budde et al., 1984; Lilford et al., 1994; Meschede et al., 2000), and several case reports with multiple affected family members have been published (Chaganti et al., 1979; Leonard et al., 1979; Shabtai et al., 1980; Cantu et al., 1981; Rivera et al., 1984; Meschede et al., 1994; Chang et al., 1999; Saut et al., 2000; Tuerlings et al., 2002), indicating that it is a potentially heritable condition.

In this article, we present a unique family with five brothers who are infertile because of severely impaired spermatogenesis. To elucidate the genetic basis for the infertility in this family, we screened all five brothers as well as their parents and two other paternally related family members for known genetic causes of impaired spermatogenesis. In addition, we performed extensive analysis of the Y chromosome, X chromosomal linkage analysis, and mutation analysis of the mitochondrial DNA.
Partial DAZ deletions in a family with five infertile brothers

Case report

A 38-year-old healthy man (Figure 1, IV-13) and his wife visited the Fertility Center Middelheim because of primary infertility for two years. Semen analysis revealed azoospermia in several semen samples. At physical examination a normal male phenotype was found. Right testicular volume was 8 mL, and left testicular volume was 10 mL. Penis, epididymides and vasa deferentia were normal. Hormonal evaluation showed a hypergonadotropic status. Bilateral testicular biopsy revealed complete absence of germ cells with normal Sertoli cells and an atrophic appearance of the Leydig cells.

The eldest brother of the proband (Figure 1, IV-10) was also known with primary infertility due to azoospermia. Therefore, we subsequently constructed a pedigree of the family (Figure 1). With permission of the index patient, all brothers and additional paternally related family members were invited to participate in the study. The index patient (Figure 1, IV-13), his four brothers (Figure 1, IV-10, IV-16, IV-17, and IV-18), their father (Figure 1, III-13), their mother (Figure 1, III-14) and two other paternally related family members (Figure 1, III-15 and IV-19) gave their informed consent. Semen analyses were performed according to the World Health Organisation guidelines and morphology was determined according to the strict criteria (WHO, 1992). With approval of the Institutional Review Board, blood samples of all individuals included in this study were collected for DNA and leukocyte isolation.

Three brothers of the index patient (Figure 1, IV-10, IV-16, and IV-17) were azoospermic, and semen analyses of the youngest brother (Figure 1, IV-18) showed severe oligozoospermia (1.8 x 10^6/ml, 44% progressive motility). Sperm count of their father (Figure 1, III-13) at age 64 was normal (67.5 x 10^6/ml, 46% progressive motility, 6% normal morphology). Semen analysis of individual IV-19 was also normal (200 x 10^6/ml, 50% progressive motility, 19% normal morphology). Individual III-15 had undergone a vasectomy so no semen sample could be obtained.

All five brothers as well as their father (Figure 1, III-13, IV-10, IV-13, IV-16, IV-17, and IV-18) had a normal 46,XY karyotype. Routine genetic screening for Y chromosome deletions, CFTR mutations and for CAG repeat length of the androgen receptor (AR) showed no abnormalities. For Y chromosome deletion screening the following STS markers were used: sY81, sY84, sY182, sY94, sY102, sY117, sY143, sY147, sY152, sY153, sY157, sY254 (table of primer sequences available on request). The paternity of the father and his five sons was confirmed by haplotypic analysis of the Y chromosome using markers YAP, M9, SRY-1532 and 92R7 (Jobling et al., 2000, 2001).

The DAZ gene copy number of all men included in the study (Figure 1, IV-13, IV-10, IV-16, IV-17, IV-18, III-13, III-15, and IV-19) was determined by analysis of sequence family variants and fluorescence in situ hybridisation (FISH). Sequence family variants that distinguish the
separate DAZ genes were detected by PCR analysis using markers SY587 and SY581 followed by restriction enzyme digestion (Saxena et al., 2000; de Vries et al., 2002). The analysis of sequence family variants showed that all men possessed only the T variant for SY581 and only the T variant for SY587. This indicates that all men tested had only one DAZ gene cluster with only DAZ3.

FISH was performed with DAZ specific probes on interphase nuclei and chromatin fibers. Cosmid 18E8 (Genbank AC010089) encompasses the 5' portions of two neighbouring DAZ genes within one gene cluster. Cosmid 46A6 (Genbank AC000022) derives from the 3' portion of the DAZ gene including exon 8-11. Probes were labelled with biotin or digoxigenin, hybridized to target DNA, and detected by avidin or antiodioxygenin antibodies conjugated to fluorochromes Cy3 (red) or fluorescein (green), respectively (Saxena et al., 2000; de Vries et al., 2002). FISH on interphase nuclei confirmed the absence of one DAZ cluster in all individuals tested. Fiber FISH showed the presence of one normal DAZ gene cluster with two genes in head-to-head orientation. This discrepancy between sequence family variant analysis and FISH has been described before and is due to polymorphism of the SY587 marker (de Vries et al., 2002).

Linkage analysis of the X chromosome of all five brothers was performed using Panel 28 of the ABI Prism linkage mapping set-MD10, version 2 (Applied Biosystems, Foster City, CA). Haplotype analysis using 10 cM spaced X chromosomal markers revealed that the five brothers do not share an identical locus on their X chromosome.

The mitochondrial DNA (mtDNA) genome of the proband, with the exception of the D-loop, was sequenced using a direct sequencing approach as described by Thorstenson et al. (Thorstenson et al., 2001). Information about the primers used for sequencing is available at the Web site of the DNA variation group of the Stanford Genome Technology center (http://insertion.stanford.edu/primers_mitogenome.html). The sequence of the index patient was compared with the revised consensus sequence (Andrews et al., 1999). Several sequence variants were identified, but all of these base-pair changes represented known polymorphisms and no new mutations. Because of the absence of mutations in the proband, no further analysis of mtDNA in additional family members was conducted.
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Discussion

Until now, a reduced copy number of the DAZ genes has only been described in individual cases of male factor subfertility and has never been detected in proven fertile semen donors (de Vries et al., 2002). In this article, we report a unique family with five primarily infertile brothers who appeared to have only one DAZ gene cluster containing two DAZ genes, instead of two clusters with two DAZ genes. Surprisingly, the father of these infertile brothers also had the same DAZ constitution, but had normal sperm counts with only a mild degree of teratozoospermia. Moreover, two other paternally related family members also had a reduced copy number of the DAZ genes. One of them (Figure 1, III-15) fathered a son (Figure 1, IV-19) who had a completely normal semen analysis.

Because the deletion is present in the father of the five affected brothers as well as in individual III-15, the deletion most likely originated in the first generation of the pedigree or earlier, and thus must be present in the other male family members in the pedigree as well. The index patient and his four brothers are the only men with proven infertility in this family, while the other eight paternally related males are fertile. As fertility is a measure of a couple and not of male gametogenesis only, we cannot exclude the presence of mild spermatogonial failure in other male family members. Unfortunately we were unable to perform a semen analysis in these other male family members.

Nevertheless, there is a striking phenotypic difference between the affected brothers and the three other family members who did participate in the study, even though they all had a reduced copy number of the DAZ genes. Apparently other factors contribute to the extreme phenotypic differences in this family. Either the fertile males have an additional factor that is not present in the five brothers, which compensates for the loss of two DAZ genes or the brothers have an additional aberration that contributes to the reduced DAZ copy number and results in infertility. This locus has to be shared by all five brothers but not by the other male family members.

One possible additional aberration could be an aberration on the X chromosome as all boys inherit the X chromosome from their mother. Because of meiotic recombination during oogenesis in the mother, the genetic constitution of the X chromosome differs between brothers within the same family. Linkage analysis in the five infertile brothers showed that they do not share an identical locus on their X chromosome, ruling out involvement of X chromosomal genes in the infertility phenotype in this family. Brothers also share their mitochondrial genome (mtDNA) because it is inherited exclusively from the mother, but we were unable to find any mutations in the mtDNA of the proband that could explain the infertility phenotype.

An third possibility could be an aberration of other genes on the Y chromosome, as there are many more multicopy genes on the Y chromosome that might be involved in
spermatogenesis. Unfortunately, at this moment, copy numbers of these genes cannot be determined.

The results of this article’s family study shows that a reduced copy number of the DAZ genes can also be present in men with normal spermatogenesis and does not necessarily lead to infertility. Consequently, partial DAZ deletions can be transmitted to the next generation spontaneously, and thus they are not always de novo deletions. The observed phenotypic differences in men with apparently identical deletions indicate that other factors contribute to impaired spermatogenesis. Furthermore, it illustrates that infertility is a complex disorder and that further research is necessary to determine the exact role of genes such as the DAZ genes in spermatogenesis.

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